

Signalling through mechanical inputs – a coordinated process

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Journal of Cell Science 125, 4172

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doi: 10.1242/jcs.119776

There was an error published in *J. Cell Sci.* **125**, 3039-3049.

Both authors should have been indicated as being authors for correspondence. The correct information is as shown above.

We apologise for this error.

Signalling through mechanical inputs – a coordinated process

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Journal of Cell Science 125, 3039–3049

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doi: 10.1242/jcs.093666

Summary

There is growing awareness that mechanical forces – in parallel to electrical or chemical inputs – have a central role in driving development and influencing the outcome of many diseases. However, we still have an incomplete understanding of how such forces function in coordination with each other and with other signalling inputs *in vivo*. Mechanical forces, which are generated throughout the organism, can produce signals through force-sensitive processes. Here, we first explore the mechanisms through which forces can be generated and the cellular responses to forces by discussing several examples from animal development. We then go on to examine the mechanotransduction-induced signalling processes that have been identified *in vivo*. Finally, we discuss what is known about the specificity of the responses to different forces, the mechanisms that might stabilize cells in response to such forces, and the crosstalk between mechanical forces and chemical signalling. Where known, we mention kinetic parameters that characterize forces and their responses. The multi-layered regulatory control of force generation, force response and force adaptation should be viewed as a well-integrated aspect in the greater biological signalling systems.

This article is part of a Minifocus on Mechanotransduction. For further reading, please see related articles: 'Deconstructing the third dimension – how 3D culture microenvironments alter cellular cues' by Brendon M. Baker and Christopher S. Chen (*J. Cell Sci.* **125**, 3015–3024). 'Finding the weakest link – exploring integrin-mediated mechanical molecular pathways' by Pere Roca-Cusachs et al. (*J. Cell Sci.* **125**, 3025–3038). 'United we stand – integrating the actin cytoskeleton and cell–matrix adhesions in cellular mechanotransduction' by Ulrich S. Schwarz and Margaret L. Gardel (*J. Cell Sci.* **125**, 3051–3060). 'Mechanosensitive mechanisms in transcriptional regulation' by Akiko Mammoto et al. (*J. Cell Sci.* **125**, 3061–3073). 'Molecular force transduction by ion channels – diversity and unifying principles' by Sergei Sukharev and Frederick Sachs (*J. Cell Sci.* **125**, 3075–3083).

Key words: Mechano-sensation, Morphogenesis, Signalling, Embryo

Introduction

Multicellular organisms require extensive coordination between cells and tissues to achieve global morphogenesis and organ function. Three major factors contribute to the induction of intracellular signalling pathways: biochemical molecules, electric currents or fields, and the application of mechanical forces (external or internal) to a tissue. During the past decades, most biological studies have focused on biochemical or electrical signalling events, for which we have now reached a thorough understanding. By contrast, despite the pioneering work carried out at the German School of Embryology at the turn of the 19th century and the influential book by D'Arcy Thompson (Thompson, 1917), the role of mechanical forces in organ formation and function did not attract full-scale attention until recently. The identification of force-induced signalling pathways in physiology (Hudspeth, 1985), the demonstration that integrins transmit forces (Wang et al., 1993) and progress in single-molecule experimentation (Finer et al., 1994; Kishino and Yanagida, 1988) have paved the way to the study of mechanical forces in biological systems. More recently, mechanical forces have been gradually revealed as major coordinators of the development and homeostasis of organisms (Janmey and Miller, 2011; Keller et al., 2003; Mammoto and Ingber, 2010).

Conventionally, 'mechanotransduction' has been defined as a process in which specific cellular machineries switch a physical

stimulus into chemical activities to trigger downstream signalling pathways (DuFort et al., 2011; Hoffman et al., 2011). Conformational changes in proteins, such as stretch-activated ion channels or mechanosensitive adhesion structures, often mediate conversion of force into chemical signalling (Moore et al., 2010). However, the definition of mechanotransduction in a global sense is not limited to the one-step switch from a force into a signal. Indeed, mechanical forces can regulate chemical signalling more indirectly, for example by altering the extracellular matrix (ECM) microenvironment or the viscoelastic properties of a cell (Kasza et al., 2007). Hence, the scope of mechanotransduction pathways should be expanded to embrace the field of 'mechanobiology'.

In this Commentary, we cover recent advances in our understanding of force-mediated events in the context of developing multicellular organisms, in particular those mediated by non-muscle myosin II. We do not elaborate on the biochemical and biophysical events involved in actin polymerization and myosin activation that have been analysed *in vitro*, nor force-induced nuclear and transcriptional events, all of which have been the topics of excellent recent reviews (Bugyi and Carlier, 2010; DuFort et al., 2011; Eyckmans et al., 2011; Hoffman et al., 2011; Mammoto and Ingber, 2010; Vicente-Manzanares et al., 2009) (see also the Commentaries in this Minifocus (*J. Cell Sci.* **125**, 3051–3060 and *J. Cell Sci.* **125**, 3061–3073). Instead, we discuss

mechanosensitive processes that involve cell shape changes or those induced by tissue growth. We pay particular attention to a macroscopic view of events on one hand, and to the viscoelastic properties of cells in their response to mechanical forces on the other hand.

Generation and regulation of forces in live organisms

Mechanical forces can be grouped into intracellular, intercellular or inter-tissue categories [for a biophysical viewpoint, see Grill (Grill, 2011)] depending on their origin in vivo (Fig. 1). As will be described in the following sections, irrespective of their category, forces that produce substantial morphogenetic effects mostly originate from the activity of contractile cytoskeletal structures (Eyckmans et al., 2011). For instance, the actomyosin cytoskeleton is responsible for cell constriction (Fig. 1A). Collective cell actions, such as cell sorting, cell migration and, indirectly, apoptosis, can all influence the behaviour of cells in neighbouring tissues by creating traction or compression forces, primarily involving the actomyosin network (Aigouy et al., 2010; Butler et al., 2009; Toyama et al., 2008) (Fig. 1B). The long-range effects exerted by muscle contractions that pull on adjacent tissues, the blood flow generated by the heart or the airway pressure created by inhaling also depend on actomyosin (Mammoto and Ingber, 2010) (Fig. 1C,D). Finally, cell-sorting events rely on the interplay between differential cell adhesion and tension (Krens and Heisenberg, 2011) (Fig. 1E). Actomyosin-independent forces also

exist, which generally involve microtubules or changes in osmotic pressure, such as in dividing cells (Stewart et al., 2011).

Mechanical forces generated in vivo resemble chemical signals in many ways. Each mechanical force possesses distinguishable features (magnitude, orientation, frequency and duration; see Box 1), which can be recognized by a specific mechano-sensing machinery. Not unlike chemical signals, these features of internal forces can be controlled and regulated by multiple mechanisms.

Generation of intrinsic forces within cells

The magnitude of contractions driven by non-muscle myosin mainly depends on the localization and activity of myosin II. Although in isolated cells various pathways can modulate myosin II activity (Vicente-Manzanares et al., 2009), in embryos the Rho-ROCK signalling pathway appears to be the main player so far (Quintin et al., 2008). Recently, the frequency and duration of contractile forces in tissue morphogenesis have attracted growing attention. They appear to display oscillatory characteristics, whereby, as first observed in *C. elegans* zygotes, myosin foci form, coalesce and then dissipate every few minutes (Munro et al., 2004). Each pulse increments a small cell shape change. Pulsed contraction patterns have been observed in a wide range of epithelial and non-epithelial cells from different species (Blanchard et al., 2010; David et al., 2010; He et al., 2010; Kim and Davidson, 2011; Martin et al., 2009) (Box 1). Several groups have recently described a new and related pulsed flow pattern of subapical myosin motors, which contributes to vertical

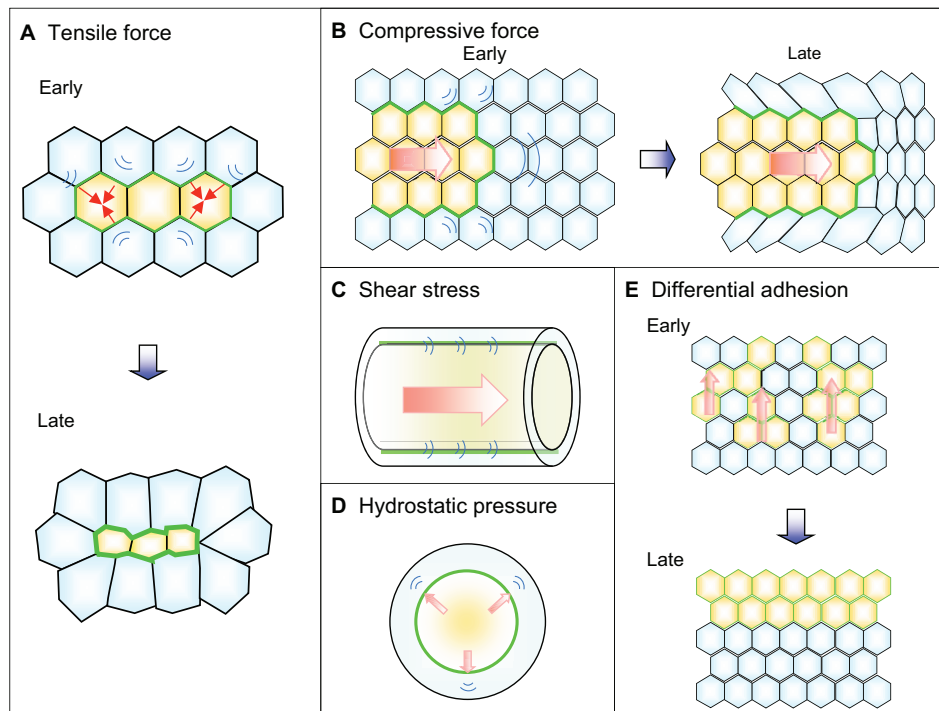


Fig. 1. Mechanisms and interfaces: of force generation in vivo. (A) Tensile force exerted by actomyosin, such as forces found in constricting amnioserosa cells during *Drosophila* dorsal closure (Solon et al., 2009). (B) Compressive force on neighbouring tissues resulting from the rearrangement or movement of large sheets, as experienced by stomodeal cells in *Drosophila* embryos during germband extension (Desprat et al., 2008) or tissue growth, and could induce the Hippo pathway (see text). (C) Shear stress that is generated by the flow of body fluid acts on surfaces of tubular tissues formed by endothelial or epithelial layers, as experienced by endothelial cells in blood vessels (Vermot et al., 2009). (D) Hydrostatic pressure exerted by a non-compressible internal tissue on a shrinking outer epithelial tubule, as observed during *C. elegans* embryonic elongation (Ciarletta et al., 2009). (E) Differential adhesion and tension between cells expressing distinct cell receptors (green and blue) as observed during vertebrate gastrulation. Classes of forces: red arrows, driving force; blue waves, resulting tensile or compressive response. Active elements are filled with yellow, responsive cells with light blue and interfaces are shown with green lines.

Box 1. Physical parameters of morphogenetic forces

Magnitude

Although force magnitude is difficult to measure in vivo, its range can be indirectly assessed. A force of 60 ± 20 nN applied with magnetic tweezers on *Drosophila* stomodeal cells induces tissue deformation that mimics the compression triggered by germband movement (Desprat et al., 2008). For comparison, a single myosin II head produces a force of 1.3–3.7 pN, whereas an early adhesion complex can sustain 100–165 pN (Moore et al., 2010) and fluid shear stress can reach more than $600 \mu\text{N}/\text{cm}^2$ in the early zebrafish heart (Vermot et al., 2009).

Average frequencies of pulsed forces

The frequency of oscillatory tissue contractions involving actomyosin foci usually spans minutes, for example, 1.4–3.8 minute in pulsed apical foci in *Drosophila* embryos (Blanchard et al., 2010; Fernandez-Gonzalez and Zallen, 2011; Martin et al., 2009; Sawyer et al., 2011; Solon et al., 2009), 1.5 minutes in *Xenopus* mesenchymal cells (Kim and Davidson, 2011) and 6 minutes in basal foci in *Drosophila* follicle cells (He et al., 2010). The frequency of oscillations involving muscle cells lies below one minute, for example, 1–3 seconds in *C. elegans* embryonic muscles (Zhang et al., 2011) and 0.6 seconds in the average heart rate of developing zebrafish embryos (Vermot et al., 2009).

Tissue stiffness

Tissue stiffness is quantified by the resistive force against tissue compression normalized by the area over which the tissue is compressed, and is expressed in Pascals (N/m^2). Embryonic tissues, such as dorsal tissues dissected from *Xenopus* gastrulating embryos, are soft, with an initial stiffness of 20 Pa, which gradually increases to 80 Pa during development (Zhou et al., 2009). For comparison, the stiffness of the softest organ, the vertebrate brain, is 100–1000 Pa and that of muscles can reach several kPa (Moore et al., 2010).

junction shrinkage during *Drosophila* germband cell intercalation (Fernandez-Gonzalez and Zallen, 2011; Rauzi et al., 2010; Sawyer et al., 2011). In contrast to cells without overt tissue planar polarity (fly mesoderm and amnioserosa), germband cells are polarized. Here, myosin II foci preferentially move towards junctions with less E-cadherin (Rauzi et al., 2010). This pulse is regulated by α -Catenin and Canoe, which promote interactions between actomyosin and adherens junctions (Rauzi et al., 2010; Sawyer et al., 2011).

The primary cause for the pulsatile nature of actomyosin foci probably reflects the dynamic instability that was observed for ensembles of motor proteins under load (Jülicher and Prost, 1995; Kruse and Rivelin, 2011). The discovery of pulsatile actomyosin foci highlights the spatio-temporal aspects of actomyosin dynamics and the role of tissue polarity cues. As discussed below, these aspects have a pivotal role in determining cellular responses.

Generation of intercellular forces by collective cell behaviours

Cell movements over large cell layers trigger tensile forces on adjacent cells and other tissue layers. Some of these movements can cause the subsequent pushing or pulling of neighbouring cells. For instance, the posterior-oriented extension of the germband in *Drosophila* compresses the stomodeum, where it triggers a mechanotransduction pathway that culminates in the expression

of *Twist*, a midgut differentiation factor (Desprat et al., 2008) (Fig. 1B). Cell proliferation along the anterior–posterior axis provides a partial driving force for *Drosophila* germband extension (da Silva and Vincent, 2007). The apoptosis of amnioserosa cells during *Drosophila* dorsal closure, combined with their apical constriction, reduces the dorsal unclosed area and pulls on the leading edge epidermis (Toyama et al., 2008). Collective cell movements can not only create a compressive or pulling force but also have a subtler role on the ECM. For instance, during *Drosophila* egg elongation, the follicle epithelial sheet surrounding the germline cyst undergoes repeated rotations along the long axis of the egg, which causes collagen IV fibrils (of up to $15 \mu\text{m}$ in length) to become oriented perpendicular to the anterior–posterior axis. Both βPS integrin (Myospheroid) and collagen IV are essential in this process because the corresponding mutants fail to undergo such rotations and have disorganized fibrils, which results in eggs that are $\sim 30\%$ shorter than normal eggs (Haigo and Bilder, 2011).

Forces generated by differential cell proliferation have a major contribution in shaping organs. For example, localized cell proliferation at the protruding bud is a key driving force for branching morphogenesis, whose occurrence is controlled by morphogenic factors, such as FGF and BMP4, in insects and vertebrates (Affolter et al., 2009). ERK1 and ERK2 (extracellular-regulated kinase) signalling in murine embryonic lungs influences spindle orientation and, in turn, the direction of the force that is generated by oriented cell division within the protruding branch (Tang et al., 2011). Furthermore, in the developing vertebrate gut, different proliferation rates between the gut tube and the anchoring tissue, called dorsal mesentery, create a twisting force that induces loop formation. Growth differences between the two tissues increases the differential strain and consequently the number of loops (Savin et al., 2011). Together, these studies highlight how different proliferation rates impact on force generation and on sculpting tissues during morphogenesis.

Mechanical properties reflected by tissue stiffness

Tissue stiffness, a measure of its rigidity, is another factor that is crucial for force regulation (for reviews, see Grill, 2011; Janmey and Miller, 2011; Kasza et al., 2007). It reflects the mechanical properties of tissues, influences the response to mechanical forces and, conversely, can also be modified by the application of force.

Tissue stiffness depends on cell density, cell size, the intracellular and cortical cytoskeleton, and ECM properties. The relationship between stiffness and cell density has been revealed by experiments measuring the stiffness of the limb-bud and flank tissues in chicken embryos. The tissue flanking the limb-budding area normally displays lower stiffness and a 50% lower cell density compared with that of the limb buds, which probably helps bud emergence. However, if cell density of the flank tissue is experimentally increased by stimulating cells with FGF8, then the tissue stiffness increases accordingly (Damon et al., 2008). A relationship between stiffness and cytoskeletal dynamics has been observed in *Xenopus* embryos, where actomyosin contractility or the depolymerisation of microtubules increases cell stiffness (Zhou et al., 2009; Zhou et al., 2010). Other factors that influence the compliance (which is the opposite of its stiffness) of a cell include integrin membrane receptors and myosin-binding proteins (Henkin et al., 2004; Lopez et al., 2005). Finally, ECM stiffness, combined with

cell spreading, modulates cell stiffness through a complex interaction. Increasing substrate rigidity from 1 to 30 kPa induces an approximately fourfold increase in cell stiffness (Tee et al., 2011), which in turn influences stem cell differentiation (Engler et al., 2006). The composition and organization of the ECM also determine its mechanical properties. A recent study using *Drosophila* organs and imaginal discs revealed that the incorporation of perlecan into an ECM containing collagen IV downregulates the constriction force that is promoted by collagen IV alone (Pastor-Pareja and Xu, 2011). A cautionary word is appropriate, however, to remind readers that cells are not homogeneous. Because cells react to a mechanical force according to its duration and magnitude, different areas of a cell might react differently within small time intervals (Janmey and Miller, 2011; Mitchison et al., 2008).

Taken together, cell-intrinsic forces can be regulated by multiple mechanisms that are often shared with conventional biochemical signal producers. Such multi-layered regulation enables force-generating machineries to produce numerous different mechanical signals with unique features. As discussed below, force-sensing machineries are able to distinguish different forces and respond appropriately.

Force-induced cellular responses and signalling cascades

Once forces are generated, they are sensed and transmitted at cell–cell, cell–ECM and cell–lumen interfaces. Junctions, adhesion structures and signalling complexes represent primary tension-sensing units, and they often display first-hand responses to forces. In recent years, the range of cellular processes identified as targets of mechanotransduction signalling pathways has steadily increased, and we will discuss some of these below.

Primary subcellular effects triggered by forces

Tension-induced pathways can either strengthen the complexes that bear tension or change the distribution of membrane receptors, primarily by inducing conformational changes of transmembrane proteins (Hoffman et al., 2011; Moore et al., 2010). As the intracellular cytoskeleton ultimately bears the impact of force application to the cells, it represents a second major class of mechanosensitive structures. The dynamic actin and microtubule filaments are classic downstream targets of tension-sensing signals (Eyckmans et al., 2011; Hoffman et al., 2011). A striking example corresponds to the formation of an actin cable within epidermal leading edge cells in response to amnioserosa cell constriction in *Drosophila* embryos (Solon et al., 2009) (Fig. 1A). Intermediate filaments, which are comparatively more stable than actin filaments, provide another potential target for mechanical forces. Phosphorylation of intermediate filaments, which is mediated by a tension-activated Rac–PAK (p21 protein-activated kinase 1) signalling pathway, promotes their recruitment to adhesion sites in the elongating epidermis of *C. elegans* embryos (Zhang et al., 2011) (Fig. 2A). Mechanical tension can also modulate intracellular trafficking (Levayer et al., 2011; Pouille et al., 2009), with a potential role for myosin II in clustering clathrin and initiating endocytosis (Levayer et al., 2011). In addition to affecting intracellular components, mechanical forces can alter the ECM itself, for instance through the reorganization of the ECM into a polarized array of collagen IV fibrils (Haigo and Bilder, 2011) (Fig. 2C). In

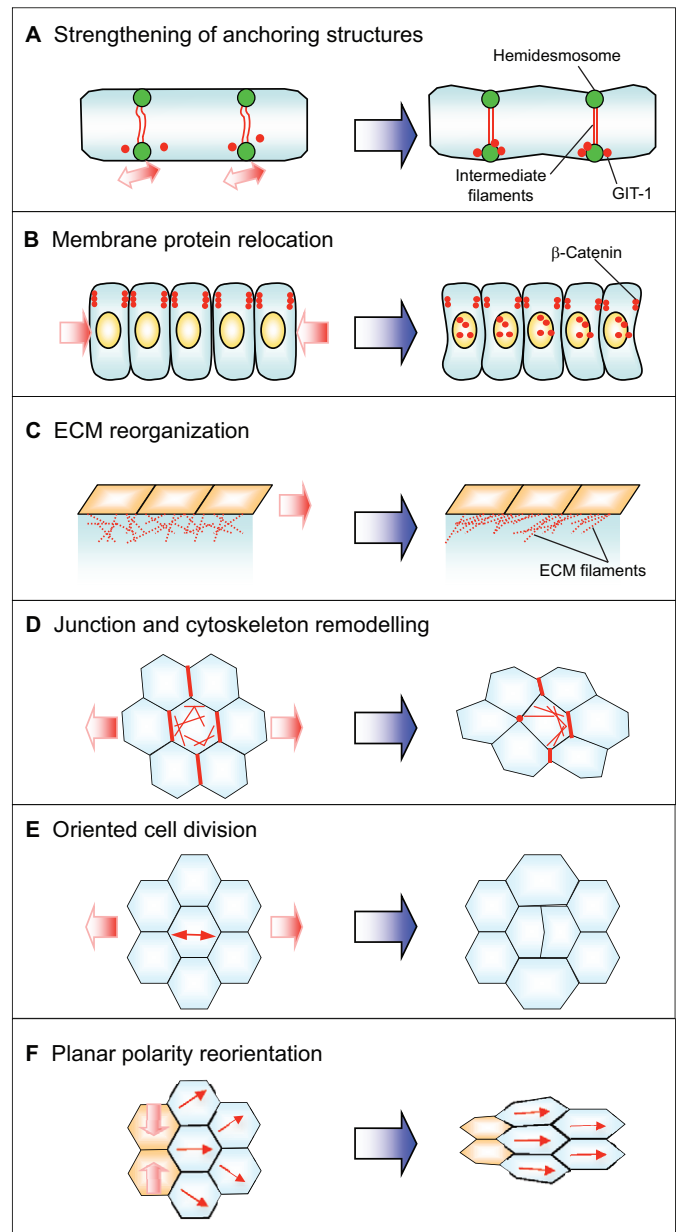


Fig. 2. Examples of force-induced cellular responses in vivo. (A) The pull of contracting muscles on the epidermis attracts additional GIT-1 to hemidesmosomes, and controls intermediate filament organization (red lines) during *C. elegans* embryonic elongation (Zhang et al., 2011). (B) Compression of epidermal cells induces the translocation of β -catenin (red dots) from the cell membrane to the nucleus in stomodeal cells that are squeezed by the extending germband (Desprat et al., 2008). (C) Collective epidermal migration remodels the underlying ECM fibrils (dotted red lines) into a polarized texture in elongating *Drosophila* eggs (Haigo and Bilder, 2011). (D) The polarized flow of actomyosin foci (thin red lines) and junctional shrinkage (thick red lines) mediate cell shape changes during germband extension in *Drosophila* (Rauzi et al., 2010). (E) An increase in mechanical stress (red arrow) triggers oriented cell division in the *Drosophila* wing blade (Aigouy et al., 2010). (F) Global tension triggered by the contraction of wing hinge cells (yellow) reorients the planar polarity (red lines) of wing blade epithelial cells (blue) (Aigouy et al., 2010). In A–F, the left part represents the starting situation and the right part the outcome after the application of force. Red arrows, direction of the force.

another example, Wnt11 downregulation in *Xenopus* blastocoel roof cells reduces their ability to exert traction stress above 0.4 N/cm² and alters fibronectin assembly (Dzamba et al., 2009).

The reinforcement and/or redistribution of membrane-bound complexes, and the reorganization of the cytoskeleton and ECM, are all immediate responses to forces. By contrast, most transcriptional activities, such as translocation of β -catenin to the nucleus (Fig. 2B), generally represent secondary responses to mechanical signals, which could achieve long-term biological effects (Desprat et al., 2008; Vermot et al., 2009).

Tension-induced global tissue organization

During morphogenesis, large-scale tissue reorganization is crucial for establishing tissue polarity and to pattern organs in their final form. Global mechanical tension has an important role in coordinating such synchronized tissue responses over long distances. In *Drosophila* embryos, an extrinsic pulling force from the invaginating mesoderm contributes to the germband extension process (Butler et al., 2009). Two recent studies report that globally oriented tension helps to establish planar cell polarity (PCP), an integrated process that impacts on cell shape change, cell division, cytoskeleton reorganization and junction remodelling (Fig. 2D,E). In the wing epithelium of *Drosophila* pupae, contraction of the wing hinge creates an anisotropic tension on the wing blade epidermal cells. This uni-directional tensile signal reorients the PCP and triggers global effects on cell elongation, cell division and junction rearrangement in the wing blade (Aigouy et al., 2010; Mao et al., 2011) (Fig. 2F). In the *Drosophila* notum, the tension generated by the shortening of indirect flight muscles affects the anterior–posterior PCP of epithelial cells. Hence, epithelial cells can respond to mechanical stress by influencing proper PCP patterning (Olguín et al., 2011).

Branching morphogenesis, a process that involves localized cell proliferation, migration and bifurcation, is probably also under the influence of mechanical tension. First, the rate of branching appears to be under the positive influence of luminal pressure through FGF signalling (Unbekandt et al., 2008). Second, a careful study of mammary gland branching using explants found that new branches initiate primarily from sites devoid of surrounding contractile myoepithelial cells (Ewald et al., 2008). This second study highlights the potential importance of tension distribution in branching, because myoepithelial cells induce local tension around the protruding branch.

Signalling cascades responsible for force-induced cellular changes

Although mechanical forces are known to exert various biological effects, the detailed signalling pathways responsible for these effects in live organisms are only now being identified. Currently, the best-described mechanotransduction pathway has been elucidated using *C. elegans* embryos. Rhythmic body-wall muscle contractions control embryonic epidermal elongation in this organism. Building on the observation that mutants with defective muscles only elongate to half their normal length (Williams and Waterston, 1994), we recently discovered that muscle contractions induce a mechanotransduction pathway through a hemidesmosome-related epidermal attachment structure (Zhang et al., 2011). This signalling cascade involves the Rac GTPase and proteins homologous to a vertebrate signalling module that includes GIT1 (G protein-coupled receptor kinase

interacting ArfGAP 1), β -PIX (also known as ARHGEF7) and PAK. Together they strengthen hemidesmosomes through intermediate filament phosphorylation and protect epidermal cells against increased tension (Zhang et al., 2011) (Fig. 3A'). Interestingly, VAB-10A, the core component of *C. elegans* hemidesmosomes, is homologous to vertebrate plectin, which, following its binding to the dystroglycan complex in alveolar epithelial cells, can relay mechanical inputs into the cell to activate protein kinases (Takawira et al., 2011). Thus, the involvement of cytoskeletal crosslinkers such as VAB-10A and plectin in mechanotransduction seems to be a common feature, although the cellular outputs vary depending on the cell types. The elongating *C. elegans* embryo thus nicely exemplifies how a contractile tissue juxtaposed to an epithelial tissue – a setting common to most vertebrate organs – can stimulate epithelial morphogenesis and differentiation.

Computer modelling and indirect evidence suggest that tension created through proliferation could provide a regulatory feedback mechanism that restricts organ growth through mechanosensitive signalling pathways (Shraiman, 2005). The evolutionary conserved Hippo signalling pathway, which regulates organ size by phosphorylating and inhibiting the transcription factors YAP1 (YES-associated protein 1) and TAZ (transcriptional coactivator with PDZ-binding motif), might have a role in this feedback. Activity of the STE20-family kinase Hippo is regulated by several actin-binding proteins, junctional proteins and polarity complexes that could sense an increase in tension caused by proliferation (for a review, see Zhao et al., 2011). In particular, the actin-binding protein Zyxin might serve as a link between mechanical tension and Hippo-regulated growth in the *Drosophila* wing disc (Hirata et al., 2008; Rauskolb et al., 2011). Interestingly, the Hippo targets YAP1 and TAZ become preferentially localised to the nucleus in large flat cells (which mimic a low-density cell population) in a process that depends on Hippo signalling, but they remain cytoplasmic in cells that are constrained to a small surface (thus mimicking high cell density) (Wada et al., 2011). Typically, the Hippo pathway helps the large trophoblast cells to adopt a fate different from that of the more compact inner cell mass in mouse embryos (Nishioka et al., 2009). Related studies agree that YAP1 and/or TAZ localization depends on cell density and actin tension (reviewed by Zhao et al., 2011). Table 1 summarizes our current understanding of the mechanosensitive signalling pathways that have been described in vivo.

Selectivity of cellular responses to different mechanical signals

Cells and tissues do not develop or function in perfect stillness. Instead, they exist in an environment that is constantly moving, which generates forces with various intensities from every direction. This raises the question as to how cells discriminate among the different mechanical inputs and whether responses remain selective under different circumstances. Preliminary answers to these issues come mainly from in vitro studies, which we briefly outline below, with comparisons to in vivo situations where relevant.

Several in vitro studies strongly suggest that cells can respond differently to mechanical inputs with different features. For instance, exposing endothelial cells to constant stretch or cyclic stretch induces different effects on growth factor expression, migration and branch formation (Zheng et al., 2008), and results

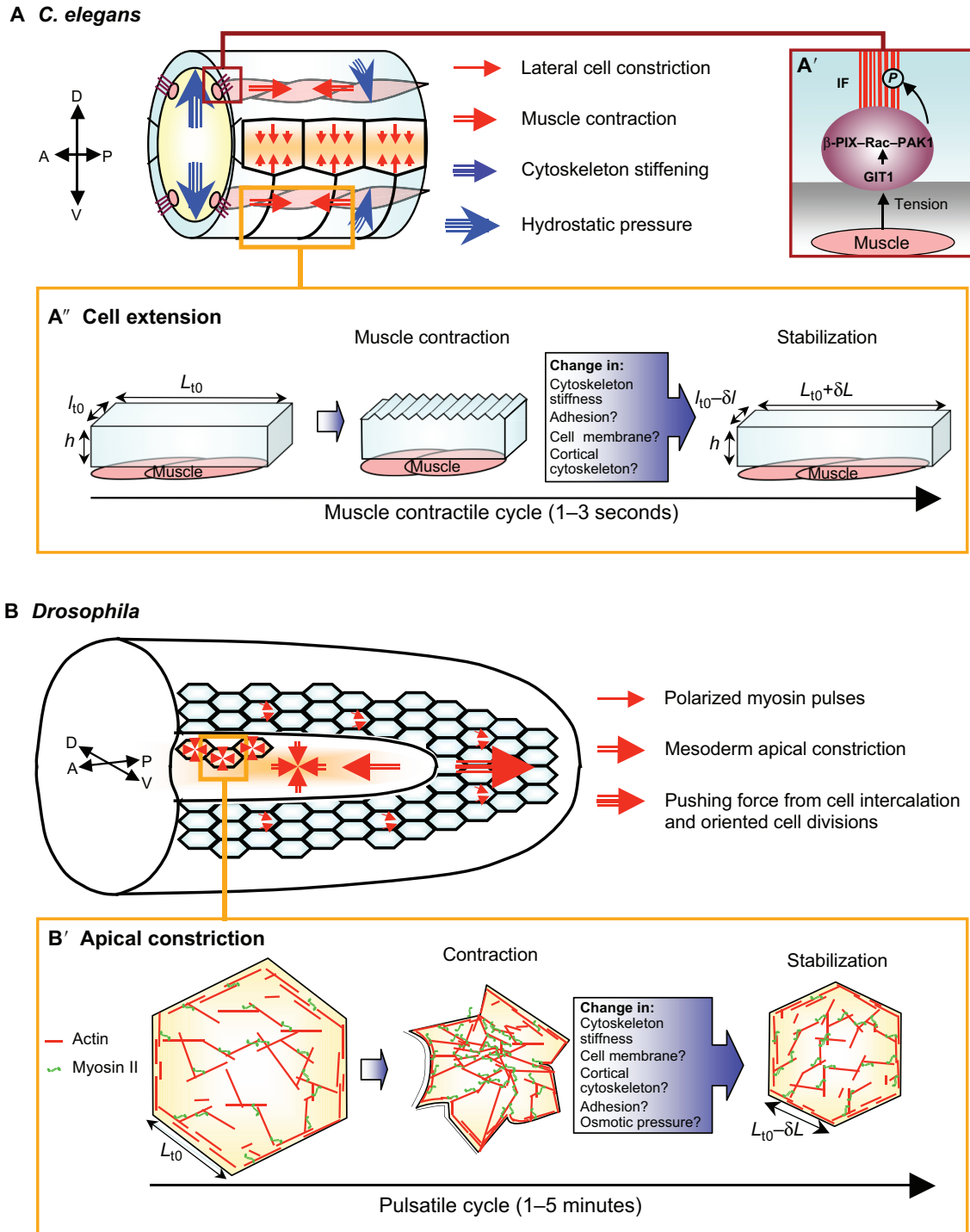


Fig. 3. The integration of multiple forces, mechanotransduction pathways and cellular adaptation during morphogenesis. (A) The four main forces exerted on the three epidermal cell rows in *C. elegans* embryos are symbolized with different arrows (Ciarletta et al., 2009). Red arrows, active contractile forces; blue arrows, resisting or static forces. (A') The force exerted by contracting muscles induces the strengthening of hemidesmosomes through a mechanotransduction pathway involving a GIT-1-β-PIX-PAK1 module. IF, intermediate filaments. (A'') The epidermal cell shape changes induced by muscle contraction trigger additional subcellular changes, some of which are hypothetical (question marks). (B) Three forces are present during germband extension in *Drosophila* embryos. Germband (blue) and mesodermal cells (yellow) undergo polarized and non-polarized apical constriction, respectively (red thin arrows), at about the same time. Mesoderm invagination impacts on germband extension. (B') During constriction, mesodermal cells experience cycles of contraction and stabilization; the contraction phase probably triggers several physical changes, mostly hypothetical (question marks). In A'' and B' changes in dimension are symbolized by the dimension L , l and h at time t_0 and after a pulse ($+\delta L$ or $-\delta L$).

Table 1. Mechanosensitive signalling events in multicellular organisms

Organism	Triggering force	Responding tissue	Mechano-sensor	Signalling pathway	Molecular target(s)	Cellular effects	Biological effects	Reference
Elongating <i>C. elegans</i> embryo	Body-wall muscle contraction	Dorsal–ventral epidermal cells	Hemidesmosomes	Rac–PIX–GIT–PAK	Intermediate filaments	Hemidesmosome strengthening; intermediate filament organization	Embryonic elongation	(Zhang et al., 2011)
Elongating <i>Drosophila</i> egg	Traction force by follicle rotation	Extracellular matrix surrounding the epithelium	ECM proteins	Unknown	Collagen IV	Fibrillar ECM organization	Egg chamber elongation	(Haigo and Bilder, 2011)
<i>Drosophila</i> embryo	Compression force by germband extension	Stomodaeal cells	Unknown	Src42A– β -catenin	Twist	Nuclear translocation of Arm (<i>Drosophila</i> β -catenin)	Midgut cell differentiation	(Desprat et al., 2008)
Gastrulating <i>Drosophila</i> embryo	Mesoderm constriction	Invaginating mesoderm	Unknown	Fog-dependent signaling	Myosin II	Apical distribution of Myosin II	Mesoderm invagination	(Pouille et al., 2009)
<i>Drosophila</i> embryo	Flow of myosin foci	Germband cells	Actomyosin and junctions	Planar polarity distinct from PCP	Myosin II	Dorso-ventral junction shortening	Germband extension	(Rauzi et al., 2010)
Embryonic zebrafish heart	Reversing blood flow	Heart valve precursor endothelial cells	Unknown	Unknown	Klf2a	Valve leaflet invagination, endothelial cell proliferation and cell shape change	Valve formation	(Vermot et al., 2009)
Chicken embryo yolk sac	Blood flow	Developing blood vessels	Unknown	Unknown	Ephrin B2; Neuropilin 1	Arterial-venous fate determination	Angiogenesis	(le Noble et al., 2004)
Mouse brain ventricle	Hydrodynamic force	Ependymal cilia	Unknown	Intracellular PCP signalling	VANGL2	Planar polarity establishment	Motile cilia orientation	(Guirao et al., 2010)

in differential induction of focal adhesion kinase (FAK) activity (Lehoux et al., 2005). Moreover, the stress fibres orient in the direction of the steady stretch but perpendicular to that of cyclic stretch (Hsu et al., 2009) with maximum efficiency at 1 Hz (Liu et al., 2008). Events in *C. elegans* offer a strikingly reminiscent parallel, because embryonic muscles contract at a frequency that is close to that inducing stress fibre realignment in vitro, and epidermal actin filaments are oriented perpendicular to the direction of muscle contraction (Box 1, Fig. 3A–A”).

In vivo, multiple forces usually act simultaneously in a given tissue, yet some responses to forces seem selective and specific (Fig. 3A,B). In zebrafish embryos, the blood flow displays a ‘forward–reverse’ pattern before the heart valves become functional. Valve precursor cells thus experience two types of hydrodynamic forces, the wall shear stress and the reversing blood flow. The development of heart valves is specifically regulated by the reversing flow, which maintains *Klf2a* expression, but not by the shear stress (Vermot et al., 2009) (Table 1). This suggests that valve precursor cells possess signalling machineries that allow them to specifically distinguish the reversing flow pattern from the shear force. It will be interesting to see whether the primary flow receptor in valve precursors is a ciliated structure or whether it involves a PCP pathway. Indeed, cilia can sense fluid shear stress (Nauli et al., 2008), promote the collective migration of the distal cell in the zebrafish pronephros and couple hydrodynamic forces to PCP signalling (Guirao et al., 2010; Nauli et al., 2008; Vasilyev et al., 2009).

Dissecting the pathways that regulate the specificity of cellular responses to force will involve a combination of genetic tools and the systematic use of emerging strategies to manually apply forces from the outside (Box 2). So far, a few examples have highlighted that external forces can partially substitute for endogenous forces. For instance, in *C. elegans*, we could partially restore hemidesmosome maturation in the absence of muscle tension by applying rhythmic compression on the entire embryo (Zhang et al., 2011). Likewise, ectopic aspiration, which mimics internal tension, can lead to recruitment of myosin II to the apical surface of intercalating *Drosophila* germband cells (Fernandez-Gonzalez et al., 2009). Manually indenting the invaginating mesoderm with a micropipette can also mimic the endogenous mechanical strain, which is absent in gastrulation-defective *snail* mutants, to promote apical myosin II recruitment, a process that is mediated by inhibiting the endocytosis of Fog (Folded gastrulation), a positive myosin II regulator (Pouille et al., 2009). Although these ectopically applied forces cannot accurately recapitulate the magnitude, orientation or frequencies of internal forces, they can still partially trigger specific downstream pathways (Desprat et al., 2008; Fernandez-Gonzalez et al., 2009; Pouille et al., 2009; Zhang et al., 2011).

Taken together, these results suggest that there are specific responses that are induced following a given range of forces. In addition, there are buffering mechanisms that can accommodate small changes in force. Deciphering the mechanisms behind the different responses will greatly facilitate our understanding of

Box 2. Monitoring, measuring and manipulating forces in live organisms

Force detection and measurement

Particle tracking nanorheology

This method quantifies the mechanical properties of multiple living cells *in vivo* by imaging and analyzing the motions of microinjected particles within the cells (Daniels et al., 2006).

FRET-based force sensors

An engineered cassette containing GFP derivatives connected to a protein domain that can be unfolded under tension. When inserted into a test protein, FRET levels can give a relative assessment of the strain (Grashoff et al., 2010; Meng and Sachs, 2011).

Tissue retraction on laser cutting

Laser beam with controlled energy is used to locally cut through cells without hurting their neighbours (Hutson et al., 2003), or through a specific cellular substructure (Rauzi and Lenne, 2011). Tension is detected by analyzing the orientation and the speed of tissue retraction.

Force manipulation

Magnetic nanoparticles and magnetic tweezer

By injecting magnetic particles into live organisms and applying a magnetic field, one can alter the tension of localized cell groups on a physiological level (Desprat et al., 2008).

Genetic mutants

Genetic mutations affecting cell contraction, migration or organ function can alter force production *in vivo* (Olguín et al., 2011; Vermot et al., 2009; Zhang et al., 2011).

External force application

Ectopic forces can be applied to organisms by compression, micropipette aspiration or indentation (Desprat et al., 2008; Fernandez-Gonzalez et al., 2009; Pouille et al., 2009; Zhang et al., 2011).

Flow manipulation

Shear force generated by fluid flow can be manipulated by perfusion or relegation of the circulation ducts (le Noble et al., 2004; Vermot et al., 2009).

how mechanotransduction pathways are integrated and coordinated in the global signalling network.

Ratchet mechanisms, elasticity and stabilization

As outlined in the previous sections, several studies have proposed that apical constriction, which is mediated by pulsatile foci, involves a ratchet process (Martin et al., 2009; Solon et al., 2009) (Fig. 3B,B'). In *C. elegans*, the epidermal cell shape changes that drive embryonic elongation involve repeated tensional inputs (Zhang et al., 2011), which is also evocative of a ratchet (Fig. 3A–A'). The possible molecular nature of the ratchet (or ratchets) represents an exciting and quite intriguing issue.

To address this issue, we must first understand how a cell that is forced to change its shape abruptly can maintain the new shape or return to its initial state. In addition to the various factors influencing cell mechanics (see the section on tissue stiffness), its viscoelasticity (i.e. the ability to recover the initial shape after a deforming stress stops) depends on the degree of stress (Fernández et al., 2006). Furthermore, microrheological approaches have suggested that the cellular and cytoskeletal

responses depend on the timescale of deformation. For timeframes below fractions of a second, the ability to recover to the initial shape depends on the elasticity of individual filaments; for those between 1 and 30 seconds, it reflects the elastic properties of the cell. For deformations applied for longer than 30 seconds, the cell might have enough time to remodel (Deng et al., 2006; Hoffman et al., 2006; Kasza et al., 2007). The frequencies of the pulsatile processes reported so far thus appear to fall into different response windows (Box 1).

Among the various factors that influence cell mechanical properties, which one could stabilize cells between two pulses? In the *Drosophila* germband, adherens junctions provide a stabilizing factor because myosin foci preferentially flow to junctions that are oriented along the dorsal–ventral axis, where their activity promotes E-cadherin endocytosis (Levayer et al., 2011; Rauzi et al., 2010). During apical constriction, shape stabilization should involve a structure that is distinct from junctions because actomyosin foci remain central. Genetic analysis of *Drosophila* gastrulation has identified two steps in the constriction process, active contraction and stabilization (Fig. 3B'). Stabilization is affected in the gastrulation mutant *twist* (Martin et al., 2009). Because Twist is a transcription factor, it must stabilize cells indirectly through its transcriptional targets. In the *Drosophila* amnioserosa, laser ablations across the tissue have suggested that it progressively becomes stiffer (Ma et al., 2009). Studies with reconstituted cytoskeletal systems suggest that the contractility of an actomyosin network requires a specific ratio of actin to crosslinker proteins and a certain threshold of motor proteins (Bendix et al., 2008). A crosslinker such as filamin A considerably modifies cell stiffening on certain substrates (Kasza et al., 2009). Hence, stabilization during apical constriction might require a modification of the ratio of actin to crosslinker to myosin. Proteins such as filamin are possible candidates to mediate such an effect, because their immunoglobulin (Ig) domains can reversibly unfold under force (Schlierf et al., 2007), which would ensure that constriction remains active after each pulse. Besides actin crosslinkers and changes in cell adhesion, the stabilization of a new shape might also involve modification of the osmotic pressure. Experiments and theoretical work have recently shown that changes in osmotic pressure, together with actomyosin, contribute to cell shape changes during mitotic cell rounding (Mitchison et al., 2008; Stewart et al., 2011). In *C. elegans*, the stabilization process is likely to involve distinct proteins, because mechanical inputs occur at a much higher frequency (Fig. 3A'). A reinforcement of hemidesmosome-like junctions is also likely to contribute to this process (Zhang et al., 2011).

Identifying the mechanisms involved in cell stabilization represents an exciting challenge for the near future, which should help reaching an integrated view of the cell response under mechanical stress.

Physical forces and chemical signalling – feedback and crosstalk

Like most biological processes, those involving mechanical forces are subject to feedback regulation. Positive feedback, negative feedback and crosstalk scenarios have all been described. In some cases, a force-producing event ‘feeds’ the assembly of a stronger force-building device through positive feedback. For instance, tension promotes the assembly of stronger myosin II minifilaments (Fernandez-Gonzalez et al.,

2009). This effect is particularly prominent during *Drosophila* dorsal closure when constricting amnioserosa cells induce the formation of an actin cable in the dorsal leading edge cells (Solon et al., 2009). This positive feedback mechanism might involve a catch-bond, which is characterized by the increased actin–myosin interaction lifetimes upon increased loads (Guo and Guilford, 2006).

In other examples, bi-directional crosstalk, such as that between *C. elegans* muscles and epidermal hemidesmosomes, has been reported. In this example, muscle contractions promote hemidesmosome integrity through a Rac–PAK pathway. Reciprocally, the loss of hemidesmosomes affects muscle structure (Williams and Waterston, 1994; Zhang et al., 2011). The tension created by increasing proliferation corresponds to a case of negative feedback in which the Hippo pathway senses tension and restricts growth (see above). Forces and biochemical signalling can also cooperate, as nicely exemplified in the *Drosophila* wing epithelium, where the global tension created by wing hinge contraction, the PCP signal triggering the accumulation of the atypical myosin Dachs at cell junctions and a polarized tension leads to oriented cell divisions that together contribute to wing blade elongation (Aigouy et al., 2010; Mao et al., 2011). Crosstalk events should have widespread influence on embryonic morphogenesis, particularly through the self-organisation of complex macromolecular structures linked to the cytoskeleton (Huber and Käs, 2011; Karsenti, 2008), as discussed above for *C. elegans* hemidesmosome biogenesis.

Concluding remarks and future prospects

The field of mechanobiology has evolved with tremendous speed over the past few years. First, a solid foundation of mechanotransduction mechanisms was built using *in vitro* cell or tissue culture systems. Then, investigations of force-induced signalling within intact organisms, empowered by inventions of cutting-edge imaging techniques and biophysics modelling approaches (Trier and Davidson, 2011) (Box 2), caught up with the developments *in vitro*. Nevertheless, several mechanotransduction pathways that have been described *in vitro* are yet to find their physiological relevance *in vivo*. Meanwhile, many force-mediated events discovered *in vivo* still await a detailed molecular explanation.

In recent years, many findings about mechanical signalling have been made in *Drosophila*. Additionally, *C. elegans* embryogenesis has also shown great potential as a model in which to study mechanosensitive events *in vivo* (Mayer et al., 2010; Munro et al., 2004; Zhang et al., 2011). The situation is more complex in higher organisms such as zebrafish, *Xenopus* and chicken embryos, but studies can be complemented by organ cultures or three-dimensional engineered tissues for which additional biophysical approaches are accessible (Davidson and Keller, 2007). Besides imaging and biophysical modelling, designing predictive models through continuum mechanics or finite element modelling should also prove powerful for advancing this field.

The field of mechanobiology, which should turn out to be particularly beneficial for medical research, combines physics and biology. From a biological point of view, we are still at a rather early age in which we ignore many of the specific developmental processes that rely on mechanical forces as well as the pathways and targets involved. Hence, drawing general rules for the response to mechanical forces might still be

premature. Although physical laws are universal, it can seem biology knows more exceptions than general rules, so from a physical point of view, the theoretical ground to explain the behaviour of biological matter might still be incomplete. Thus a lot remains to be discovered and there are exciting times ahead in the field of mechanobiology.

Acknowledgements

We apologize to our colleagues whose work could not be cited owing to space constraints. We are grateful to Christelle Gally and Julien Vermot for critical reading of this manuscript.

Funding

Work in IGBMC is supported by grants from the Agence Nationale pour la Recherche, Association pour la Recherche sur le Cancer and institutional funds from CNRS, INSERM and Université de Strasbourg.

Note added in proof

Several papers of direct relevance to the issues discussed in this Commentary have been published since the final acceptance of the manuscript, and we would like to briefly mention two.

First, Roh-Johnson, Goldstein and colleagues (Roh-Johnson et al., 2012) suggest that before the junction and apical membrane can actually move a molecular clutch should engage pre-existing actomyosin contractions. Their work identifies the Rac GTPase, one of its upstream regulators and E-cadherin as potential actors or regulators of the clutch. It refines and potentially challenges the ideas illustrated in Fig. 2D. Second, Bosveld, Bellaïche and collaborators (Bosveld et al., 2012) have examined the patterns of cell proliferation and tissue deformation in the fly dorsal thorax. They report that areas where the PCP protocaderin Dachsous mediates the polarised accumulation of the atypical myosin Dachs in turn anisotropically build up tension, which leads to changes in tissue shape. This study illustrates how PCP can lead to tissue deformation (see also Fig. 2F) and nicely illustrates the power of systematic image analysis coupled with physical modelling.

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